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Preparation and Properties of Cnidocytes from the Sea Anemone *Anthopleura elegantissima*

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Abstract. Cnidocytes were isolated from the tentacles and acrorhagi of *Anthopleura elegantissima* by enzymatic treatment with papain followed by centrifugation in a Percoll-containing medium to produce a concentrated fraction of these cells. The morphology of the isolated cells, as revealed by light and electron microscopy, showed that these cells had intact plasma membranes and was comparable to that of cells *in situ*. Comparison of the ability of different substances to induce discharge in isolated and *in situ* cnidocytes showed that the responsiveness of isolated cells was reduced, but not abolished, compared to *in situ* cnidocytes. Electrophysiological recordings made from cnidocytes isolated from acrorhagi showed that these cells possess voltage-activated ionic currents, further proof that the isolation procedure did not effect the integrity of the plasma membrane. Discharge did not occur with changes in membrane potential.

Introduction

Cnidocyte is a collective term for the various types of "sting cells" used for prey capture, defense, and other functions by members of the phylum Cnidaria. Their common feature is a specialized product of the golgi apparatus, the cnida (or capsule or cnidocyst), which contains a coiled, inverted tubule. With appropriate stimulation the enclosed cnida discharges; the inverted tube rapidly everts into the external environment and either entangles the prey or penetrates it and injects a venom. The classification of cnidocytes is largely based upon the architecture of the cnida and its tubule and divides these cells into three major catagories: nematocytes, spirocytes, and ptychocytes (for reviews see Mariscal, 1974, 1984).

An understanding of the physiology of cnidocytes (for reviews see Picken and Skaer, 1966; Mariscal 1974), has been handicapped by the fact that *in situ* these cells frequently form part of a complex tissue. Thus it is difficult to separate the actions of the cnidocyte from those of the various accessory and supporting cells. The development of techniques for recovering isolated, intact cnidae from various sources (for review see McKay and Anderson, 1988), and the ease with which this can be done, made isolated cnidae the subject of numerous physiological and biochemical studies. These studies provide a detailed understanding of cnida structure, biochemistry, toxicology, and the mechanics of discharge. Isolated cnidae provide little useful information, however, on the physiological control of discharge since cnida discharge ultimately involves a stimulus to the cell's membrane which is absent in isolated cnida. Thus, a functional, intact membrane is necessary if a thorough understanding of the control of cnida discharge is to be achieved. Therefore, we were interested in developing methods with which to study cnida discharge using isolated, functional cnidocytes.

Previously we reported a method for isolating cnidocytes from *Cladonema* sp. (Hydrozoa), *Chrysaora quinquecirrha* (Scyphozoa), (Anderson and McKay, 1987), and *Hydra littoralis* (Hydrozoa) (McKay and Anderson, 1986, 1987) and described some of the electrophysiological properties of those cells. Here we report a substantial modification of that method that makes it possible to isolate large numbers of intact, functional cnidocytes from sea anemone tissues. We also compare the ability of isolated and *in situ* cnidocytes to discharge under a variety of conditions.

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Materials and Methods

Animals

Specimens of *Anthopleura elegantissima* were obtained from the Marine Science Institute, University of California, Santa Barbara, and were kept in flowing seawater at 15–18°C. Animals were fed twice weekly on a diet of *Artemia* nauplii, fish eggs, and shrimp.

Chemicals and solutions

Papain was obtained from Cooper Biochemicals and Percoll was purchased from Pharmacia. CHAPS (3-[3-Cholamidopropyl)-dimethylammonio]-1-propanesulfonate), deoxycholic acid, and taurocholic acid were purchased from Sigma. All other chemicals were reagent grade and obtained from Fisher Scientific. The different salines and their compositions were as follows: Ca-free artificial seawater (m*M*), NaCl, 476; KCl, 9.7; $MgCl_2$, 24; $MgSO_4$, 27; $NaHCO_3$, 3; HEPES, 10; pH 7.4, artificial seawater (m*M*) NaCl, 466; KCl, 9.7; $CaCl_2$, 10; $MgCl_2$, 24; $MgSO_4$, 27; HEPES, 10; pH 7.4 and concentrated artificial seawater (m*M*), NaCl, 1120; KCl, 22; $CaCl_2$, 23; $MgCl_2$, 65; $NaHCO_3$, 2.

Cnidocyte preparation

Animals were anesthetized in a 1:1 mixture of seawater (SW) and 0.37 *M* $MgCl_2$. When insensitive to touch, the tentacle ring was removed with scissors and transferred to a smaller vessel containing the same medium. Individual tentacles and acrorhagi were separated from other tissue and placed in 1.5 ml polypropylene microcentrifuge tubes. It is crucial that mucus produced during the dissection be removed and kept to a minimum.

Cnidocytes were freed from the surrounding tissues by enzymatic digestion with papain prepared as follows. 0.05 ml of a papain suspension in 50 m*M* sodium acetate buffer (28.8 μg/μl; 20.9 BAEE units/mg) was added to 0.95 ml SW followed by the addition of 0.2 mg of dithiothretol (DTT). The pH was adjusted to pH 7.5–7.6 with NaOH. The tissue was added to the enzyme solution and the volume adjusted to 1.5 ml with SW. The ratio of volumes of medium to volumes of loosely packed tissue was approximately 4:1. The tissue was then placed on an oscillating shaker whose speed was just sufficient to keep the tissue in motion. The progress of the digestion was followed by microscopic examination. Usually 1–1.5 hours were required to liberate large numbers of cnidocytes, although longer times were sometimes necessary. When the tissue was judged sufficiently dispersed, the whole digest was centrifuged on a Fisher Microcentrifuge equipped with swinging buckets for 3–4 minutes at an RCF of 1100 × *g*. This pelleted all of the dispersed cells without undue compaction. The supernatant was discarded and replaced with 0.72 ml of Percoll and 0.48 ml of concentrated artificial SW. The pellet was resuspended by gentle tapping and allowed to sit several minutes until most of the tissue had risen towards the surface. The tube was then centrifuged again at an RCF of 2000 × *g* for 5 minutes. Following centrifugation unwanted cells and debris remained in the upper portion of the medium. The supernatant was discarded and the pellet, composed almost exclusively of cnidocytes, was resuspended in a small volume of SW. In some cases it was necessary to resuspend the pellet and recentrifuge it in the Percoll-containing medium to remove excess debris.

Scanning electron microscopy (SEM)

Isolated cnidocytes were allowed to adhere to protamine sulfate-coated coverslips, rinsed to remove nonadhering cells and fixed as previously described (Anderson and Schwab, 1984). After fixation, specimens were critical-point dried with CO_2, sputter-coated with gold-palladium, and examined using a JEOL 35C.

Transmission electron microscopy (TEM)

Isolated cnidocytes were fixed using the same protocol as for SEM except that the isolated cnidocytes were fixed as a suspension. Solutions were exchanged by briefly centrifuging the cells into a pellet, replacing the supernatant, and then resuspending the pellet. After fixation the isolated cnidocytes were dehydrated through an ethanol series and propylene oxide and embedded in Epon. Prior to polymerization, the cells were centrifuged into a pellet to make it easy to locate them for thin sectioning. Sections were post-stained with uranyl acetate and lead citrate and examined with a JEOL 100 TEM.

Cnidocyte behavioral assay

The ability of isolated cnidocytes and *in situ* cnidocytes in the tentacles of *A. elegantissima* to discharge under a variety of conditions was assayed in the following manner. Excised tentacles were pinned to a layer of Sylgard (Dow Corning) in the bottom of a petri dish containing approximately 5 ml of SW or artificial saline. Isolated cnidocytes were allowed to settle on and adhere to coverslips coated with protamine sulfate. Test substances, in solution, were applied from a micropipette using brief (5–100 ms) pulses of N_2 (Picospritzer II, General Valve Co.). The pipette was positioned to within 10 μm of the cell being tested. With this system we ejected volumes from 20–840 × 10^{-9} l. The results were observed through a Hoffman Modulation Contrast microscope (total magnification = 320×).

Electrophysiological recordings

Electrophysiological recordings were obtained from cnidocytes isolated from the acrorhagi. Whole-cell patch clamp recordings were made using a Dagan 8900 Patch Clamp Amplifier equipped with a 0.1 Gohm headstage. Patch pipettes were filled with a high K^+, low Ca^{++} recording solution (Anderson, 1985). Leakage and capacative currents were removed from the records presented here by subtracting currents generated by equal and opposite voltage steps (for details of the methods, see Anderson and McKay, 1987).

Results

Nomarski micrographs of aliquots of cnidocytes are shown in Figure 1. The bulk of material is intact, isolated cnidocytes; the amount of debris is small. The majority of cnidocytes isolated from the tentacles (Fig. 1A) are spirocytes, although nematocytes are also present. The cnidocyte membranes appear intact and the cnida is visible within each cell. We were unable to separate the different types of cnidocytes from the tentacles as they all have a very low buoyant density beyond the density (d > 1.12 g/ml) we could conveniently achieve using Percoll solutions. Cnidocytes isolated from the acrorhagi are shown in Figure 1B. Again the cnida is visible within each cell. Most of the cell cytoplasm appears as a small girdle or bleb encircling the cnida.

The appearance of the isolated cnidocytes was better revealed using SEM. (Fig. 2). What is clearly a spirocyte is shown in Figure 2A. The membrane has collapsed around the tough cnida with its uneverted tube. The nematocyte (type unknown) in Figure 2B shows a protoplasmic extrusion (arrow) from its apical end; this may be a ciliary structure. Such structures were observed on only a few cells.

To further verify that the isolated cnidocytes were intact cells we examined their ultrastructure by transmission electron microscopy (Fig. 3). It should be stressed that these sections were obtained from pellets of isolated cnidocytes; hence, other cells are present. Figure 3 shows a nematocyte in cross-section. The cell's interior is dominated by the cnida. The inverted tube is embedded in an electron-dense material and is surrounded by the thick two-layered cnida wall, which in turn is surrounded by the cnida membrane. The nucleus and other cytoplasmic structures are visible also and all are enclosed by an apparently intact plasma membrane.

A spirocyte in longitudinal section is shown in Figure 3B. The cnida wall is single-layered and the internal surface has the serrated appearance characteristic of spirocytes (Picken and Skaer, 1966.). The cnida and other cytoplasmic constituents are once again surrounded by a plasma membrane.

Electrophysiology

Although electron microscopy showed that the isolated cnidocytes were intact, we made electrophysiological recordings to demonstrate that their membranes were functional. Because of their relatively large size we used cnidocytes isolated from the acrorhagi. Under voltage clamp (Fig. 4A), the total membrane currents evoked by step-depolarizing the cell from a holding potential of −70 mV through a range of depolarized membrane potentials consist of both inward and outward currents: a fast inward current followed by a more prolonged outward current. A current/voltage plot for the peak inward current (Fig. 4B) shows that the inward current activates at approximately 0 mV, reaches a maximum at +20 mV, and reverses at +65 mV. We did not study the ionic dependence of this or any other currents in these cells.

Observations on in situ *and isolated cnidocytes*

The treatments given to excised tentacles and isolated cnidocytes and their efficacy in evoking discharge are summarized in Table I. To assess the effect the extracellular medium may have had on a cnidocyte's ability to discharge, treatments were repeated in three different media: Ca-free ASW, normal SW, and 1:1 0.37 *M* $MgCl_2$ and SW. Because spirocytes comprise the majority of cnidocytes in anemone tentacles and can be unambiguously identified in the undischarged state, they were the only type of isolated cnidocyte tested. We did not attempt to identify the types of cnidocytes that discharged in the excised tentacle.

In situ cnidocytes bathed in normal SW invariably were discharged by 0.5 *M* KCl. Isolated cnidocytes bathed in normal SW responded only occasionally to 0.5 *M* KCl; there were no responses in the other two bathing media. When calcium was added to the 0.5 *M* KCl (final [Ca] = 0.17 *M*) *in situ* cnidocytes discharged in the Ca-free artificial seawater (ASW) as did a few of the isolated cnidocytes bathed in Ca-free ASW and normal SW.

Acid and alkaline SW were only minimally effective at eliciting discharge. Only *in situ* cnidocytes bathed in SW and "spritzed" with acidified SW (HCl, pH 2.5) discharged. To test for osmotic effects, distilled water and 1 *M* sucrose in SW were tested. Neither substance caused discharge. Since calcium chelating agents have been implicated in the discharge of isolated cnidae (Lubbock and Amos, 1981), we examined the effect of the sodium citrate and potassium citrate. Only potassium citrate evoked discharge and only in *in situ* cnidocytes bathed in normal SW.

To further examine the role of external ions in controlling discharge, we examined the efficacy of 0.5 *M* KCl in discharging *in situ* cnidocytes in bathing solutions of differing ionic compositions. In Na-free (N-methyl-D-

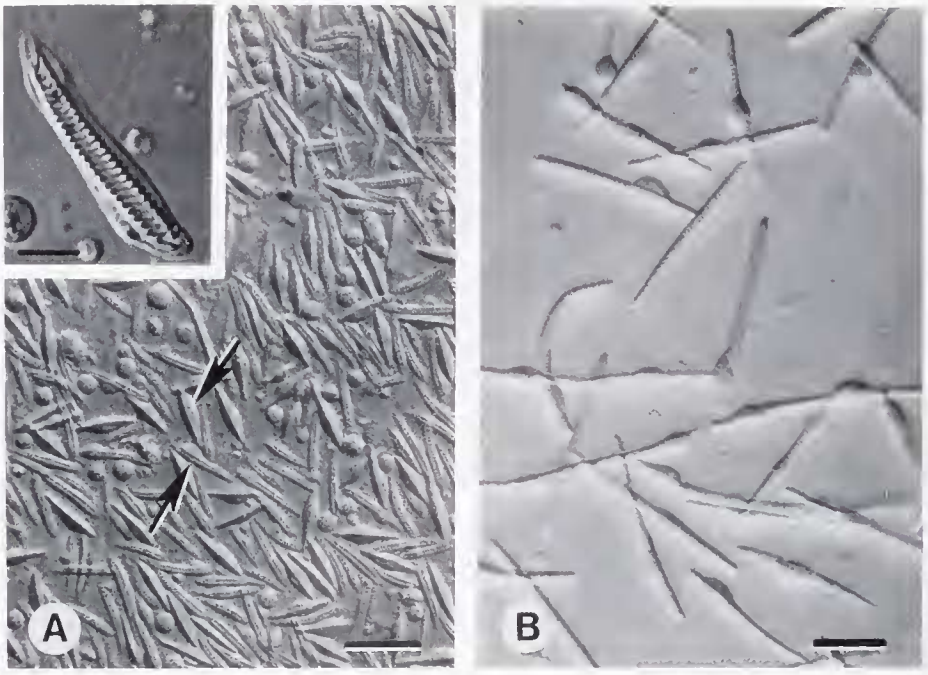

Figure 1. Nomarski micrographs of representative aliquots of cnidocytes isolated from tentacles and acrorhagi of *Anthopleura elegantissima*. (A) Isolation of cnidocytes from tentacles produced a mixture of cnidocyte types. Arrows show different kinds of cnidocytes (scale = 25 μm). Insert is a higher magnification view of a spirocyte isolated from the tentacles. The coiled tubule of the cnida is clearly visible (scale = 5 μm). (B) Cnidocytes isolated from acrorhagi (scale = 50 μm).

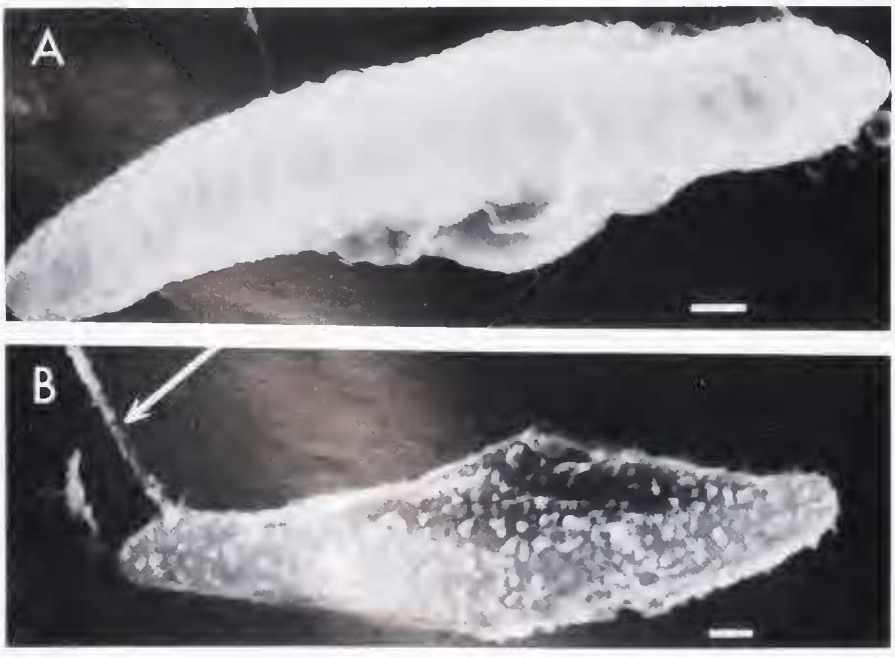

Figure 2. Scanning electron micrographs of two different types of cnidocytes isolated from the tentacles. (A) Spirocyte. (B) Nematocyte, type unknown. The cytoplasmic projection (arrow) may be a ciliary structure (A, B, scale = 1 μm).

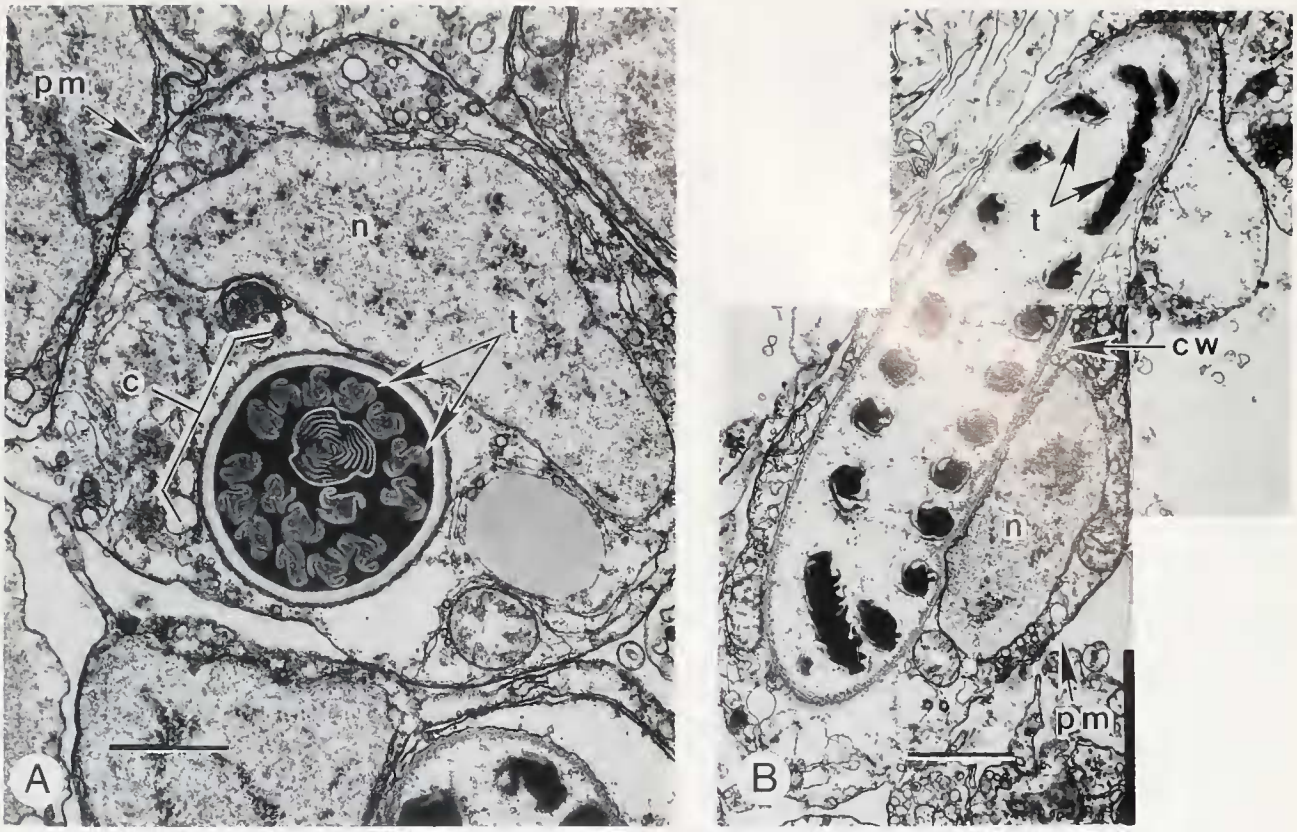

Figure 3. Transmission electron micrographs of different types of cnidocytes isolated from the tentacles of *Anthopleura elegantissima*. The fixed cells were embedded as pellets. (A) A cross-section of a nematocyte containing an undischarged cnida in its cytoplasm (scale = 1 μm). (B) Longitudinal section through a spirocyte with an undischarged cnida and other cytoplasmic organelles (scale = 2 μm). c, cnida; cw, capsule wall; n, nucleus; pm, plasma membrane; t, tubule.

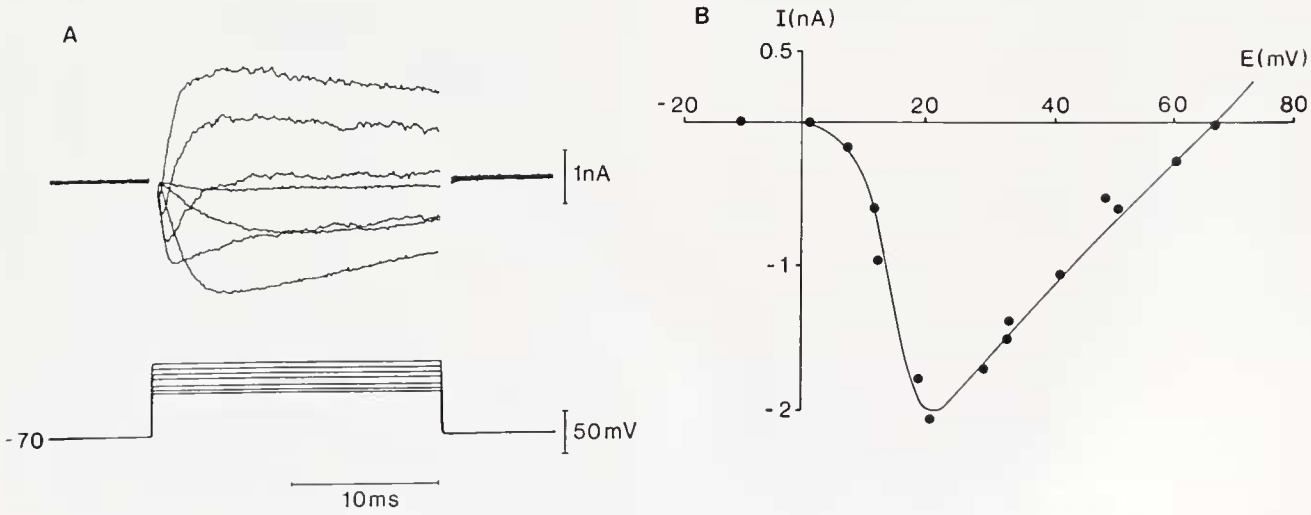

Figure 4. Voltage-clamp recordings from a cnidocyte isolated from the acrorhagi of *Anth... gantissima*. (A) Record of the total membrane currents produced when the cell was step depolarized from a holding potential of −70 mV to −20 mV and then in 7.5 mV increments. (B) Current-voltage plot for the peak inward currents obtained from (A).

Table I

Comparison of the ability of different substances to discharge isolated and in situ *cnidocytes from* Anthopleura elegantissima. *Whole tentacles (WT) and isolated spirocytes (IS) were tested in three different bathing solutions*

	WT	IS	WT	IS	WT	IS
Bathing medium	Normal SW		Ca-free ASW		(1:1) 0.34 *M* $MgCl_2$/ SW	
Test solution						
0.5 *M* KCl	+	+/−	−	−	−	−
0.5 *M* KCl + 0.17 *M* $CaCl_2$	+	+/−	+	+/−	−	−
SW pH 2.5	+	−	−	−	−	−
SW pH 10.5	−	−	−	−	−	−
Potassium citrate 100 m*M*	+	−	+	−	nt	nt
Sodium citrate 100 m*M*	−	nt	−	nt	nt	nt
Taurocholic acid 25 m*M*	+	+	+	+	+	+
Deoxycholic acid 25 m*M*	+	+	+	+	+	+
CHAPS 25 m*M*	+	+	+	+	+	+
1 *M* sucrose in SW	−	−	−	−	−	−
Distilled water	−	−	−	−	−	−
Brine shrimp homogenate	−	−	−	−	−	−
Fish skin homogenate	−	−	nt	nt	nt	nt

Key: + discharge; − no discharge; nt not tested.

glucamine substituted) or Mg-free (Na substituted) ASW, discharge occurred in response to applied KCl. Discharge to applied KCl was, however, inhibited by the addition of the potassium channel blocker, 4-aminopyridine (7.5 m*M*) to a bathing solution of normal SW, implying that potassium is acting through blockable channels.

The only substances that consistently elicited discharge in both isolated and *in situ* cnidocytes in all media were derivates of the cholic acids (CHAPS, taurocholic and deoxycholic acids). These substances have been used classically to demonstrate the competence of cnidocytes (Pantin, 1942) and isolated cnidae (Burnett *et al.*, 1968) to discharge, but considering the strong lipid solubilizing nature of these substances it is not surprising that they are effective discharge inducing agents.

To investigate more physiologically realistic stimulants, homogenates of *Artemia* nauplii and fish-skin (*Mugil cephalis*) were prepared by sonicating these tissues in SW. The filtrates of these substances had no effect on either isolated or *in situ* cnidocytes. (Table I).

Discussion

We have developed a method for isolating intact, functional cnidocytes from the tentacles and acrorhagi of a sea anemone using enzymatic dissociation. This procedure enabled us to isolate intact cnidocytes in a fraction that was essentially free of other types of cells. The technique appears to be generally applicable to cnidocyte-bearing tissues. Using the same basic method, with modifications, we have been able to dissociate cnidocytes from a variety of other cnidarians, including *Aiptasia* sp., *Bunodosoma cavernosa, Calliactis tricolor* (Anthozoa) (unpub. results), *Chrysaora quinquecirra* (Scyphozoa), *Cladonema* sp., and *Hydra littoralis* (Hydrozoa) (McKay and Anderson, 1986, 1987; Anderson and McKay, 1987).

Enzymatic dissociation of cnidocyte-bearing tissues has been attempted previously. Pepsin (Glaser and Sparrow, 1909), papain (Phillips and Abbott, 1957), and trypsin (Yanagita, 1959) were all reported to be either ineffective or to have a deleterious effect on the condition of the cnidae. The reason for the difference in success is unclear, but it could be attributable to the type of enzymes used since we found that papain from different sources produced variable results; some were essentially ineffective while others produced only isolated cnidae.

A variety of approaches were used to determine whether the isolated cells were intact and to distinguish between complete cells and isolated cnidae. Light microscopy clearly demonstrated that the bulk of the isolated tissue was composed of cnidocytes, as defined by the presence of the cnida. In many cases (Fig. 1A, insert) organelles, particularly nuclei, could be seen between the cell membrane and the cnida, suggesting that the cells were intact. The presence of the cell membrane and various organelles was confirmed in electron micrographs of thin sections of pellets of isolated cnidocytes (Fig. 3). The apparent absence of ciliary cones on our isolated cnidocytes was worrisome and may reflect damage during the isolation procedure. In anthozoans, however, ciliary cones occur only on nematocytes and not spirocytes (Robson, 1973; Mariscal *et al.*, 1976) so their absence on all cnidocytes is not unexpected.

The presence of the cell membrane and other organelles suggests that the cells are intact but ultrastructural techniques provide only limited information on the health of the cell. Although the isolated cnidocytes retained the ability to discharge, this is not proof that they are in good condition because isolated cnidae can discharge. The fact that the isolated cells supported transmembrane ionic currents (Fig. 4A) and negative resting potentials is good evidence that their membranes were intact and functional and not damaged by the isolation procedure.

A further reason for conducting electrophysiological experiments with these cells was to determine whether discharge could be elicited by electrical activity. A common means of effecting discharge from isolated cnidae and intact tissues is with electrical stimulation (Lubbock

et al., 1981). Previous work (Anderson and McKay, 1987) on cnidocytes from hydrozoans and scyphozoans has shown, however, that cnida discharge cannot be effected by changes in membrane potential nor by the presence or selective blockade of any of the ionic currents present in the cell. The fact that none of the cells examined electrophysiologically in this study discharged during the recordings indicates that this finding can be extended to the anthozoans. The reason why electrical stimulation is such an effective means of evoking cnida discharge is, therefore, unclear although it is possible that the type of electrical stimuli required to elicit discharge may be physiologically unrealistic.

The difficulties of inducing spirocyte discharge has been noted before (Robson, 1973) and our results agree with this, but taken together, our results show that although the responsiveness of isolated cnidocytes to stimuli is greatly diminished compared to *in situ* cnidocytes, it is not abolished. This parallels the sensitivity of isolated cnidae as compared to *in situ* cnidocytes (Blanquet, 1970).

Previous work (Conklin and Mariscal, 1976) on the responsiveness of cnidocytes *in situ* has shown that cnidocyte discharge requires a combination of chemical and mechanical stimuli. In that type of study, the typical approach was to coat a glass rod or coverslip with the chemical stimulant and then to place it in contact with the cnida-bearing tissue, thus providing both a chemical and mechanical component to the stimulus. However, working with single cells meant that we could not use the traditional methods of mechanical stimulation. Instead we attempted to stimulate the cells by spritzing a known chemical at the cell; the force of ejection served as the mechanical component. The use of brief, discrete episodes of stimulation reduced the risk of receptor desensitization from prolonged exposure to the stimulant. Although we could quantify the chemical component of the stimulus, this could not be done with the mechanical component. Indeed, a poor mechanical stimulus may explain the failure of the brine shrimp or fish skin homogenates to discharge either the *in situ* or isolated cells since both these tissues, when intact, are readily captured by the tentacles of intact anemones.

While the low incidence of discharge could be partly attributable to the quality of the mechanical components of the stimulus, the fact that so few isolated cnidocytes discharged may also mean that *in situ* their discharge is regulated by adjacent cells and nerves, as has been proposed elsewhere (Westfall *et al.* 1971; Westfall, 1973; Hufnagel *et al.*, 1985).

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